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San Francisco

Separation of Native Proteins and Protein Complexes Using CESI-MS

James A. Wilkins Ph.D.
Mass Spectrometry Facility

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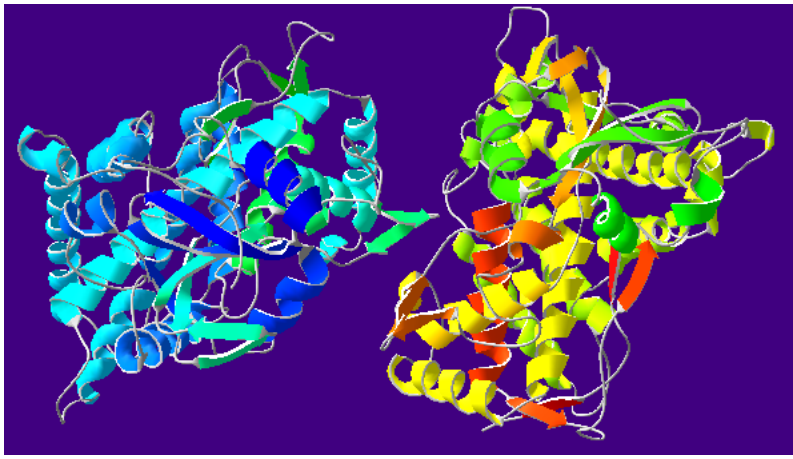
Native Protein Separations by CESI-MS Monitored by Mass Spectrometry

Advantages

- Direct coupling of capillary electrophoresis separation with mass spectrometry detection
- Separations by charge and size
- Greatly expanded molecular weight range compared to alternative approaches such as size exclusion chromatography
- Potential for "fine-tuning" separations using novel capillary coating chemistries

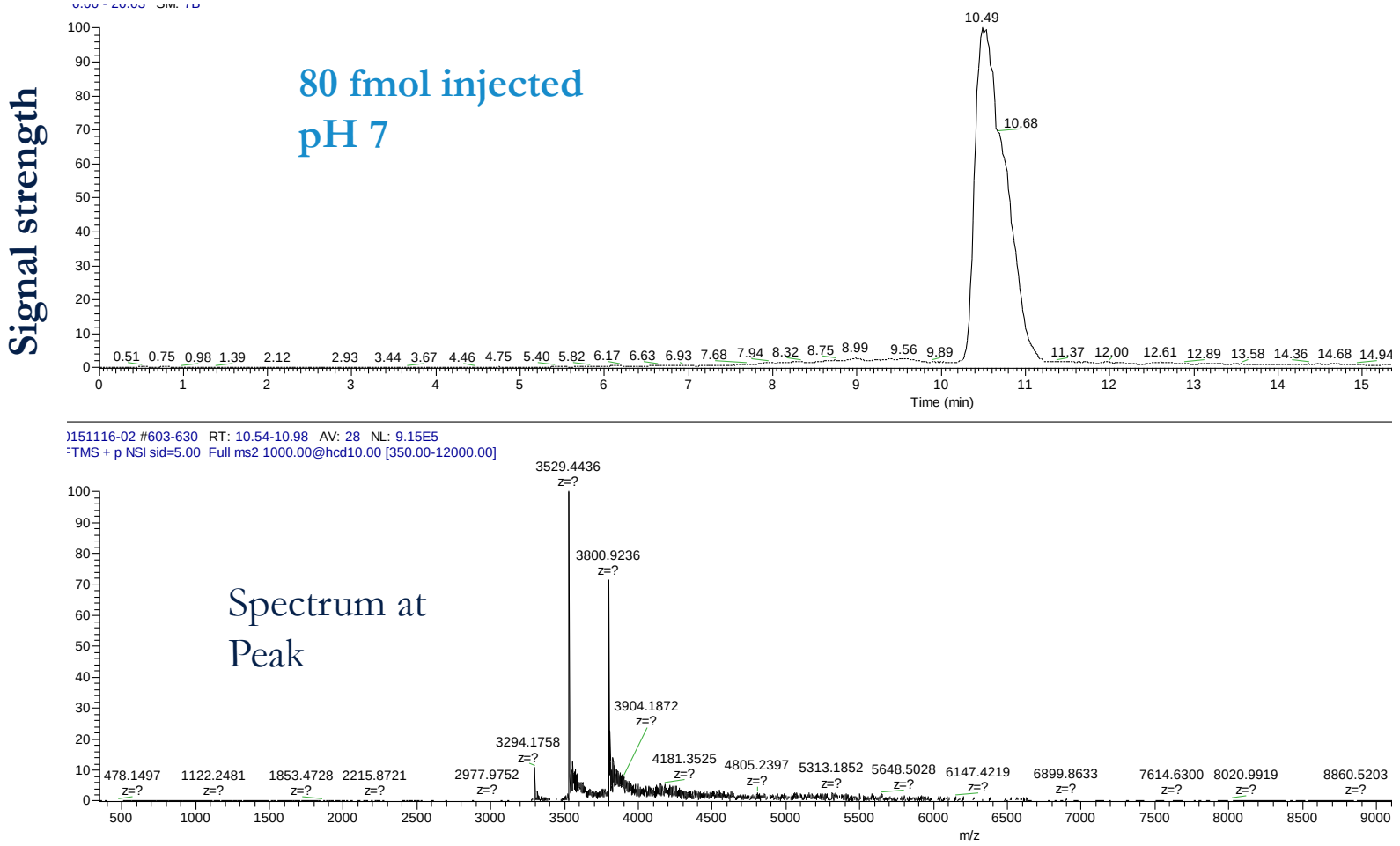
Native Protein Separations by CESI-MS Monitored by Mass Spectrometry

Examples

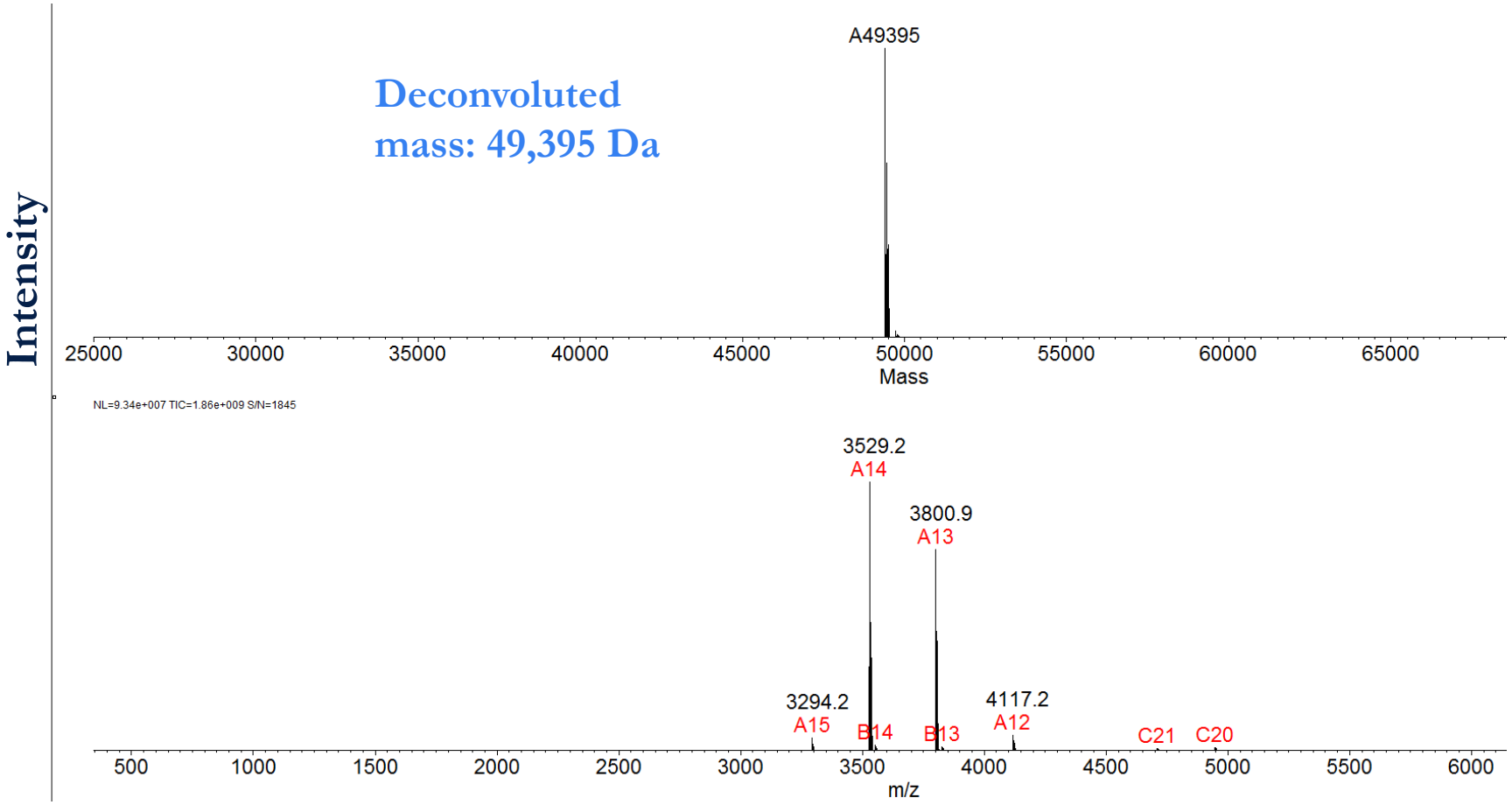


1. **Cytochrome P450 (Cyp 124)**
   - Represents one of 20 different enzymes present in *Mycobacterium tuberculosis*
   - Cytochrome P450s represent potential therapeutic targets in treatment of Tuberculosis
   - Use CESI-MS to detect interactions of Cyp 124 with ligand

Cyp 124 (a Cytochrome P450 Derived From *Mycobacterium tuberculosis*)



Cyp 124 Deconvoluted Spectrum



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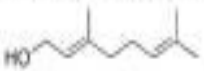
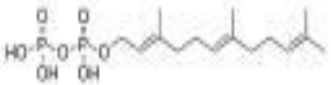
Cyp 124 Ligands

Compound	Chemical structure	Dissociation constant K_D, μM	Specific activity*	Michaelis constant K_M, μM
Lauric acid		>100†	n.d.	n.d.
Palmitic acid		>100†	0.07 ± 0.03†	n.a.
15-Methyl palmitic acid		1.01 ± 0.07	7.6 ± 1.5	9 ± 4
Phytanic acid		0.22 ± 0.006	9.9 ± 2.7	54 ± 8
Arachidic acid		n.d.	n.d.	n.d.
Phytane		205 ± 14	n.d.	n.d.
Pristane		178 ± 18	n.d.	n.d.
Geraniol		25 ± 1.8	n.d.	n.d.
Farnesol		1.04 ± 0.05	15.5 ± 2.8	36 ± 3
Geranylgeraniol		0.48 ± 0.06	9.6 ± 3.1	32 ± 4
Farnesyl diphosphate		90 ± 13	4.8 ± 0.9	n.a.

From Johnston et el (2009) PNAS 106, 20687-20692

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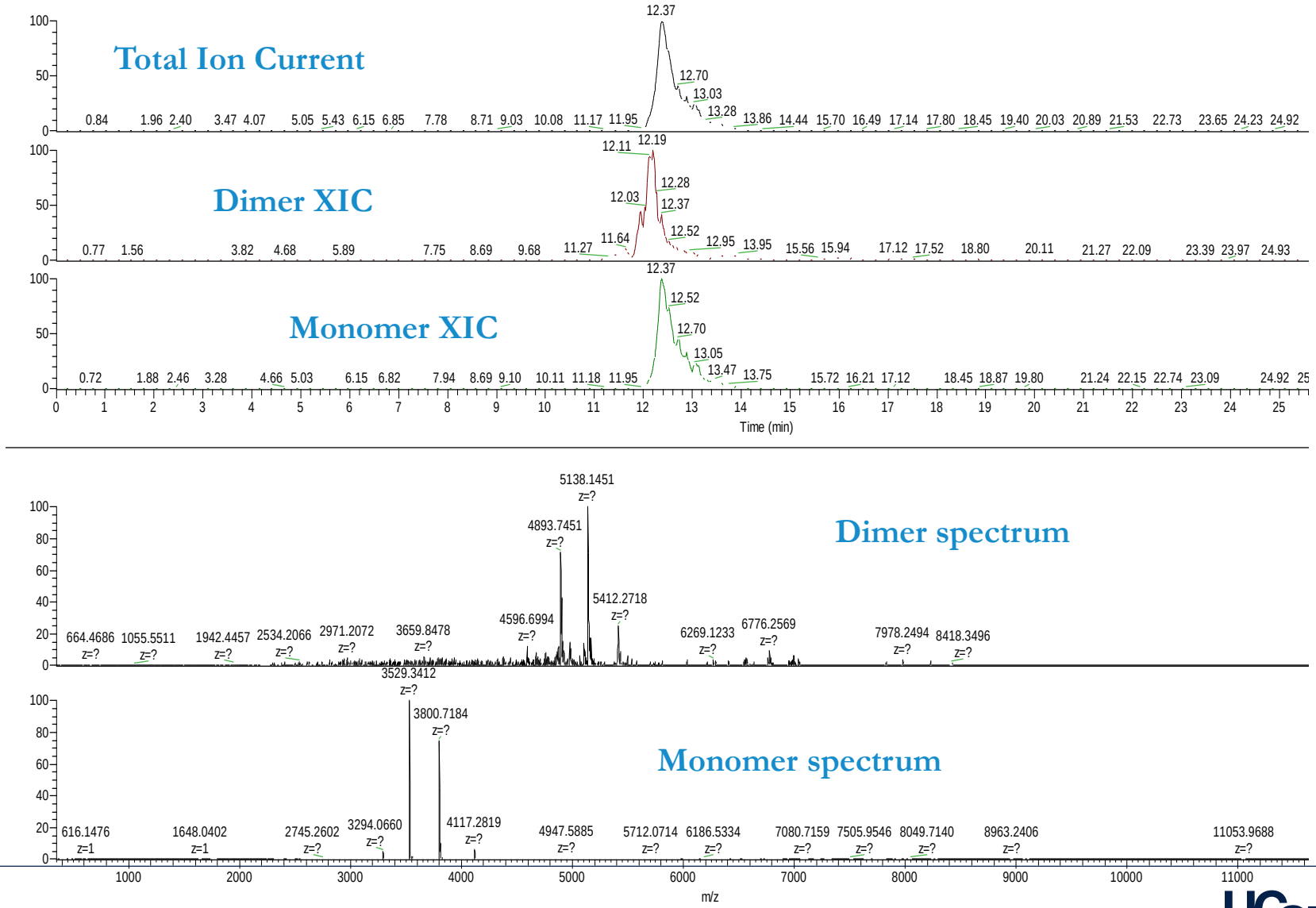
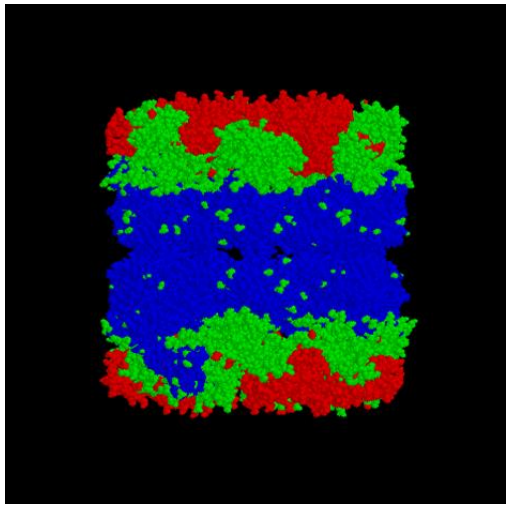
Cyp 124: Farnesol-induced Dimer Separation From Monomer BGE: 100 mM Ammonium Acetate, pH 7



Examples

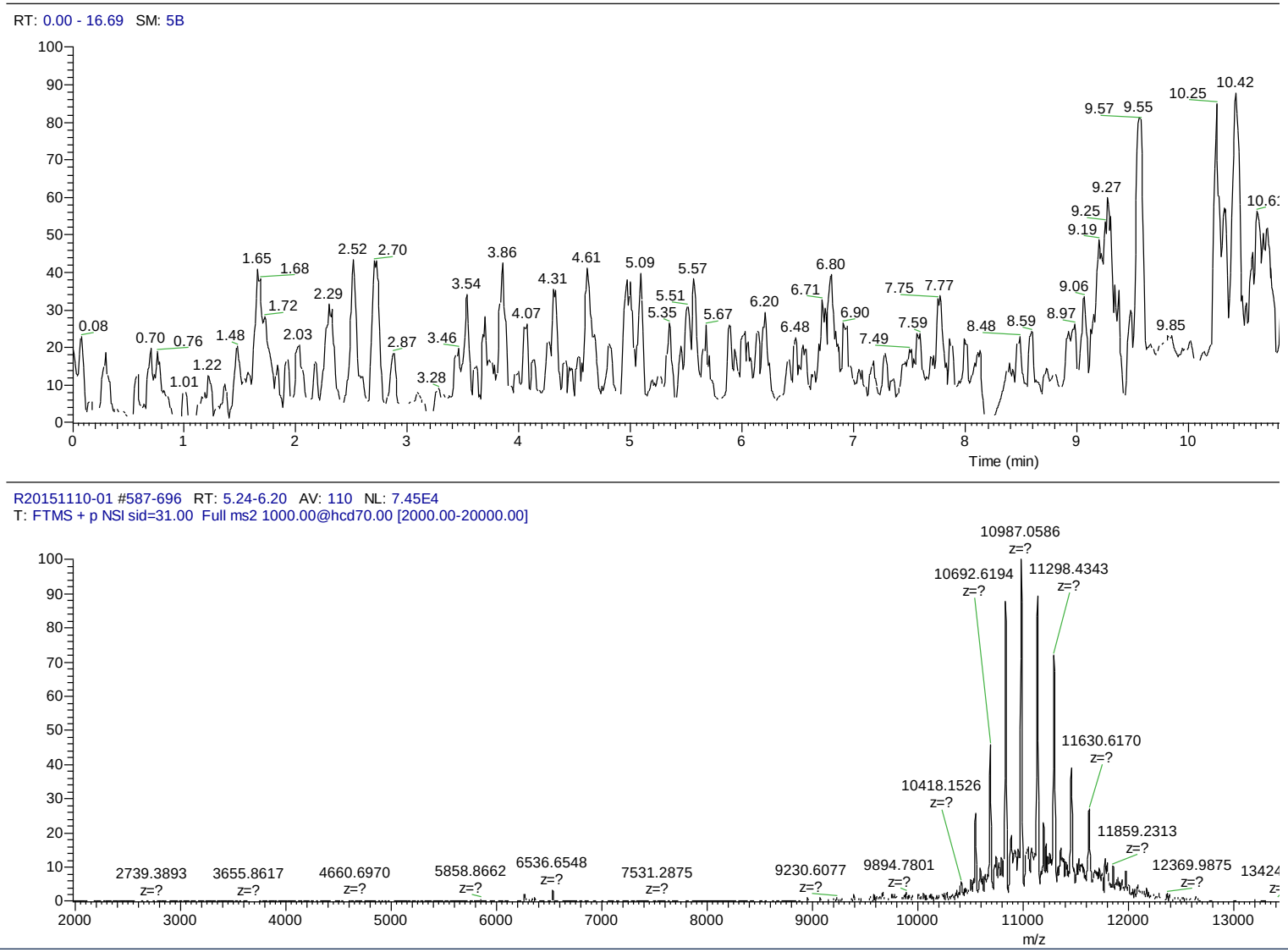
1. **Cytochrome P450** (**Cyp 124**): represents one of 20 different enzymes present in *Mycobacterium tuberculosis*. Cyp's represent potential therapeutic targets in treatment of Tuberculosis

2. **GroEL**
   - A molecular chaperone, 14-subunit protein complex from *E. coli* and other bacteria.
   - Native mass is approximately 800 kDa.
   - Use CESI-MS to probe conformational changes.

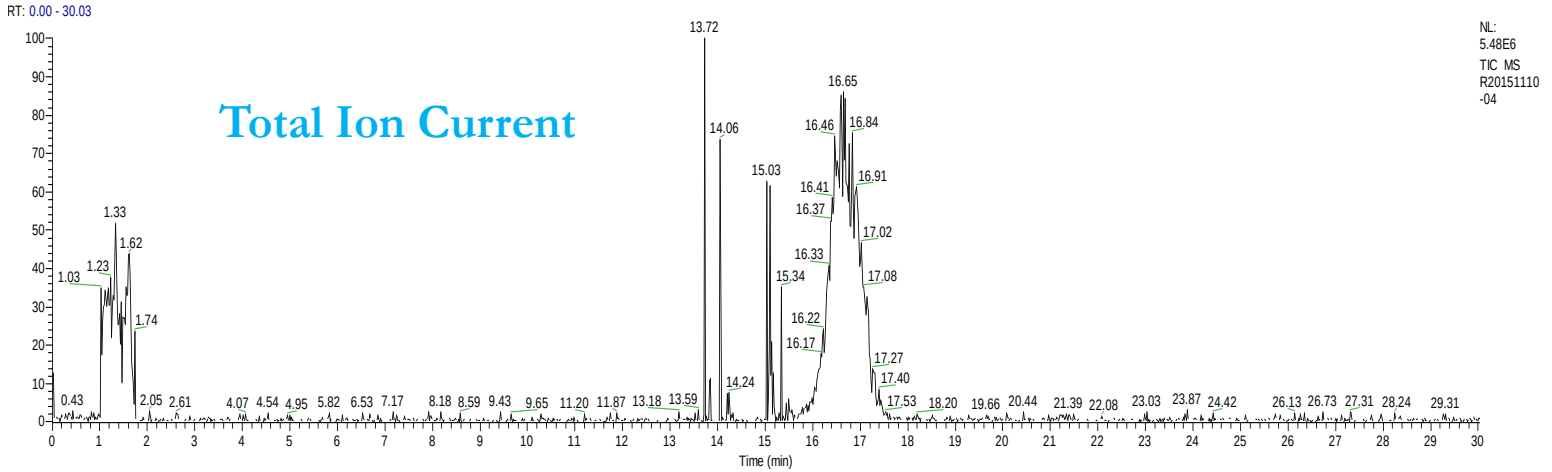


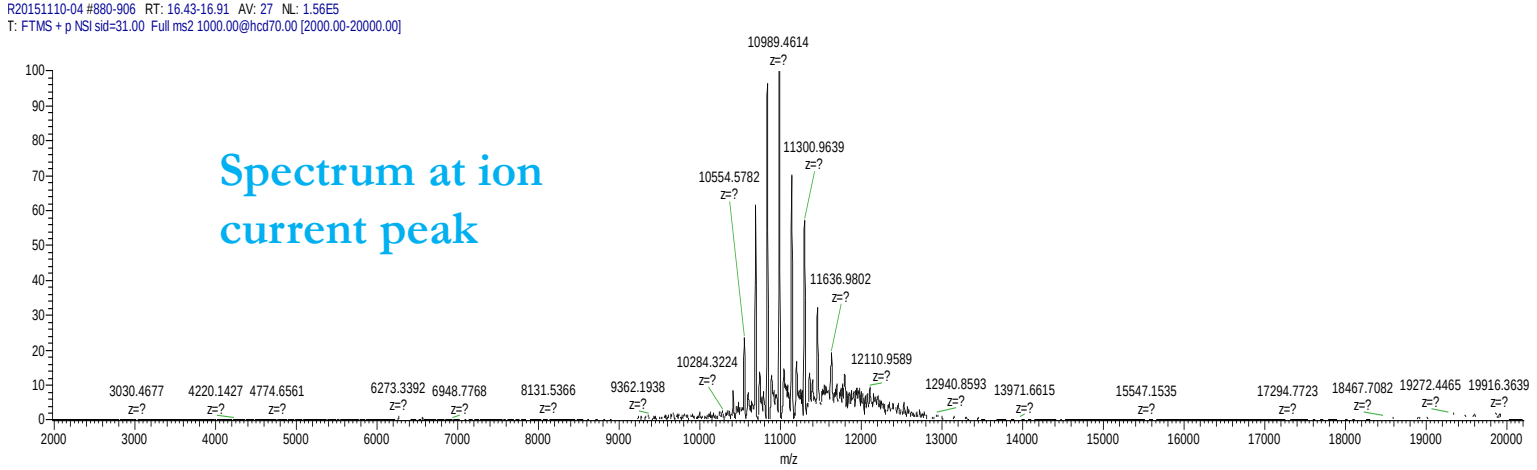
GroEL (side view)

GroEL Infusion

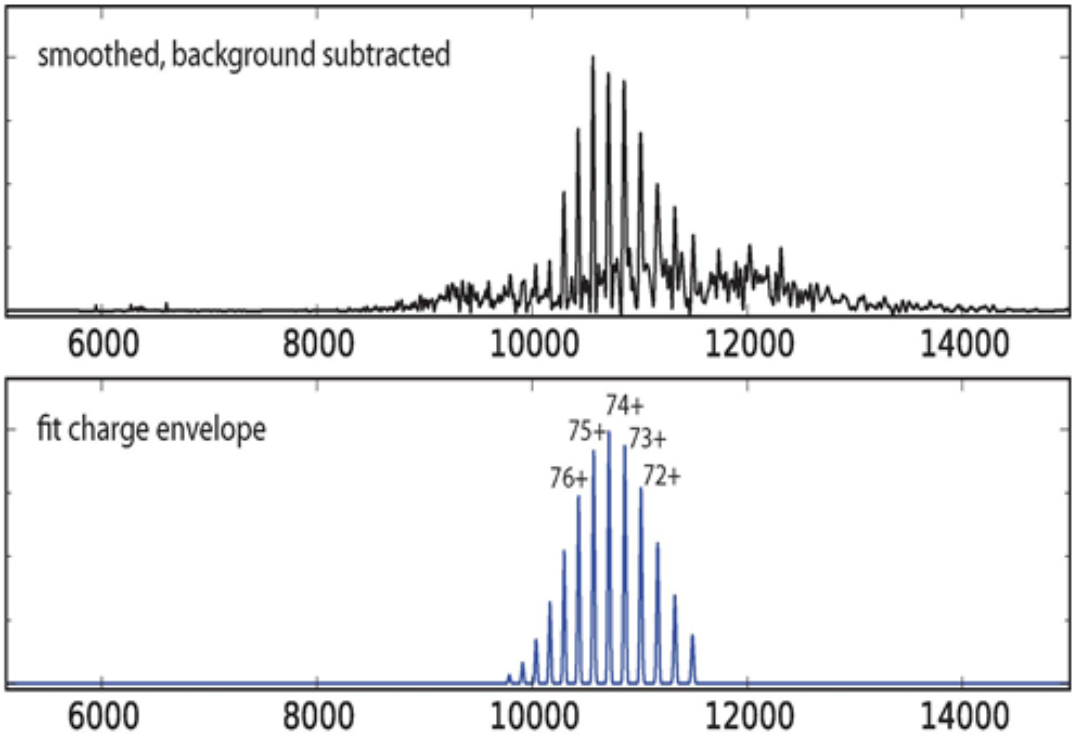


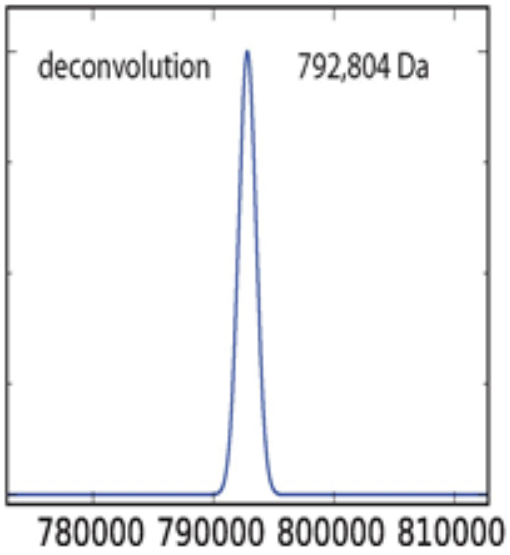
CESI-MS Run – GroEL
BGE: 100 mM Ammonium Acetate, pH 7





GroEL Deconvoluted Spectrum From CESI-MS Peak





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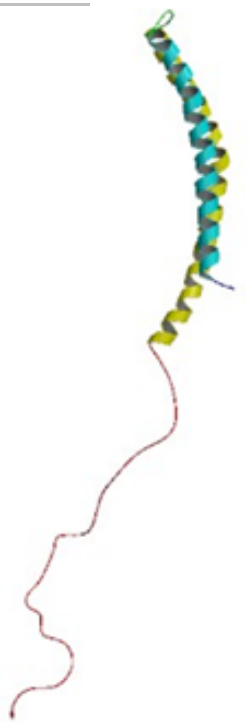
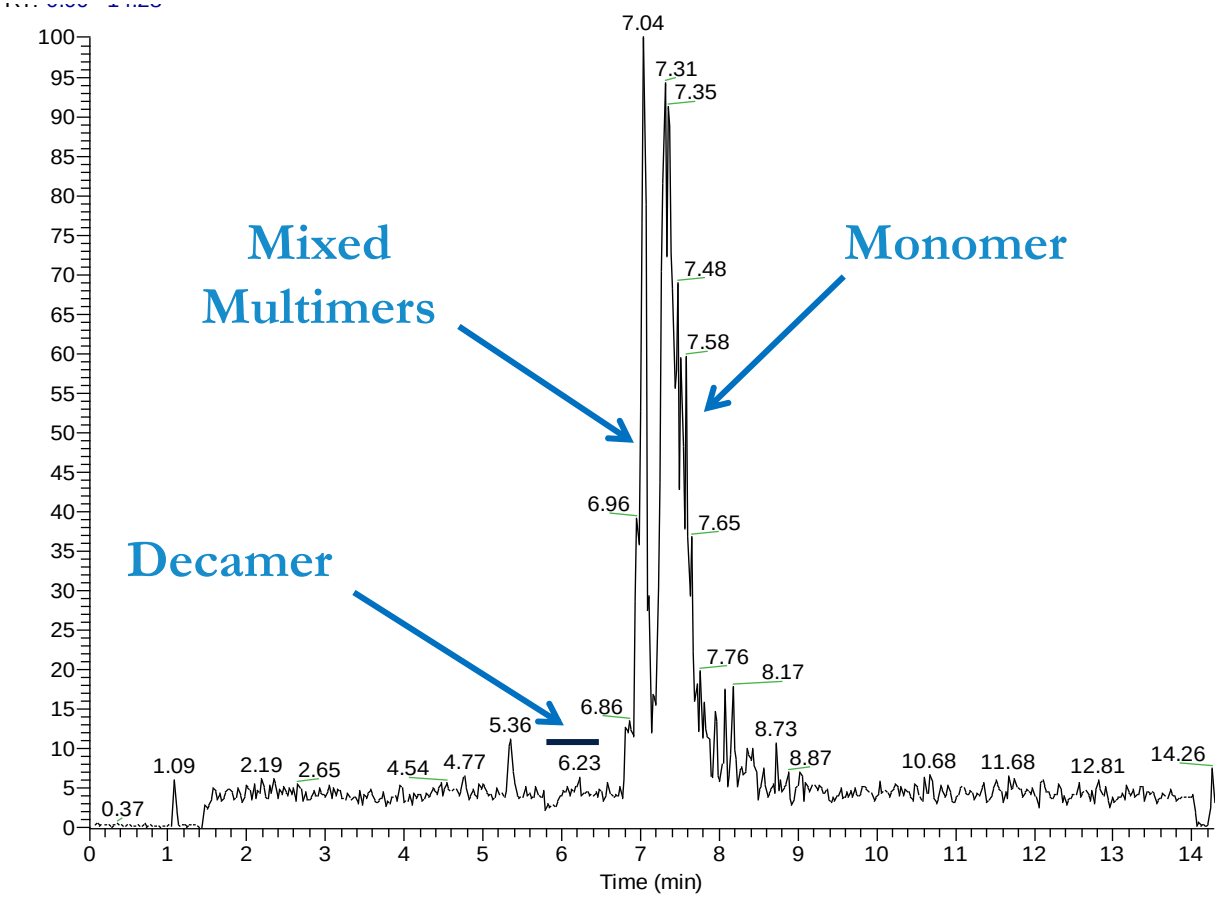
Examples

1. **Cytochrome P450 (Cyp 124)**: represents one of 20 different enzymes present in *Mycobacterium tuberculosis*. Cyp's represent potential therapeutic targets in treatment of Tuberculosis
2. **GroEL**: a molecular chaparone, 14-subunit protein complex from *E. coli* and other bacteria. Native mass is approximately 800 kDa.
3. **Alpha-synuclein**

- A presynaptic ~14kDa protein, strongly implicated in the pathology of Parkinsons Disease (PD).
- Aggregated forms are found associated with Lewy Bodies, the hallmark lesions in PD.
- Use CESI to detect and separate aggregate forms of the protein.



Total Ion Electropherogram of Partially Aggregated Alpha Synuclein Sample



CESI shows separation of multimeric and monomeric forms of the amyloidogenic protein *a-synuclein* under native conditions (100 mM ammonium acetate, pH 7) as detected by mass spectrometry

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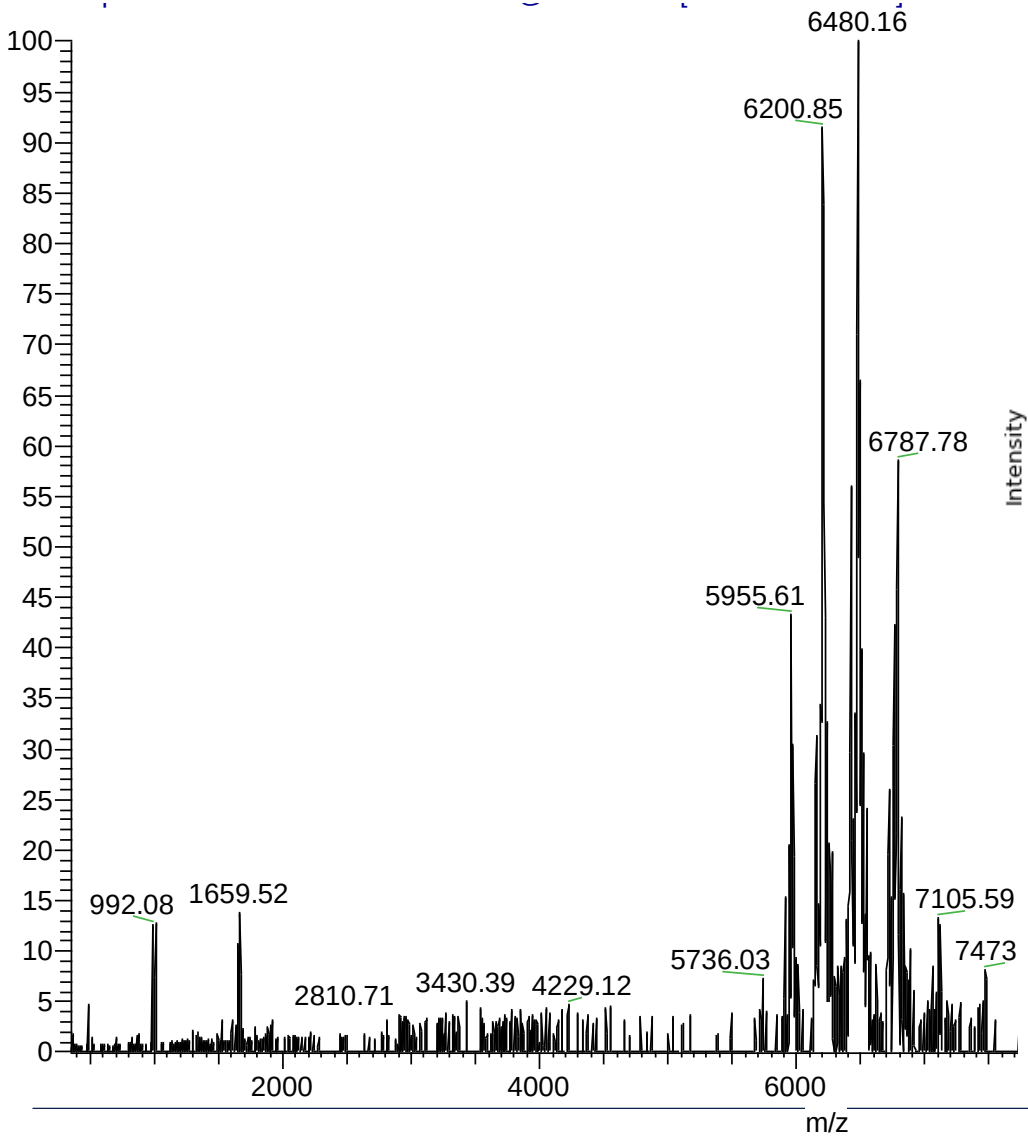
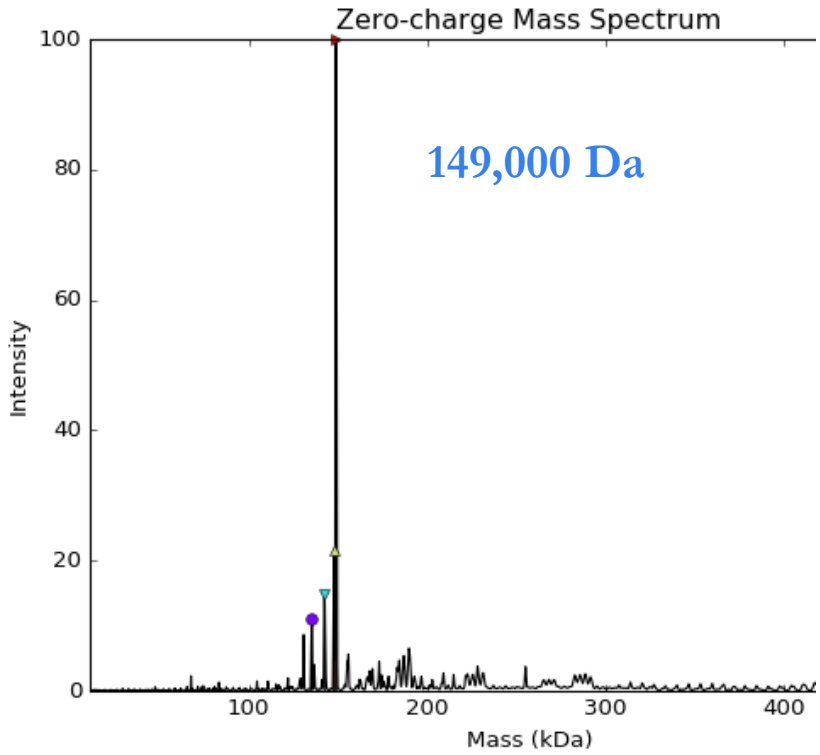
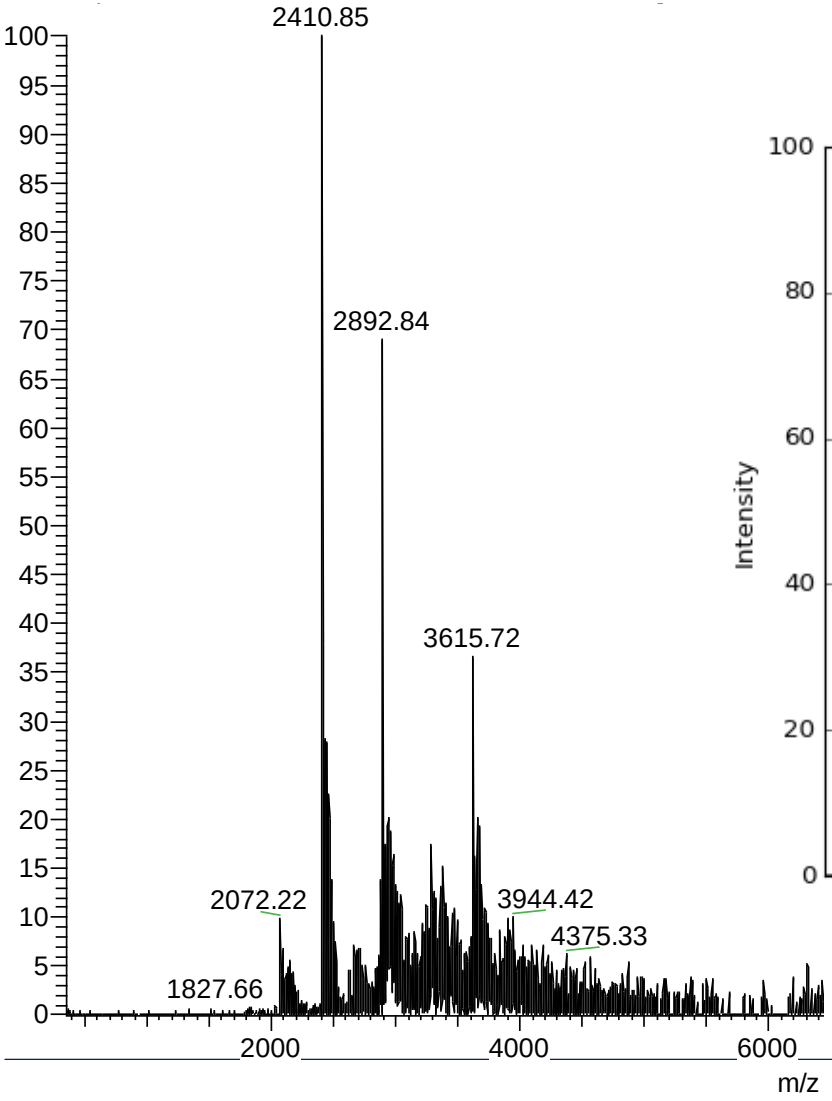
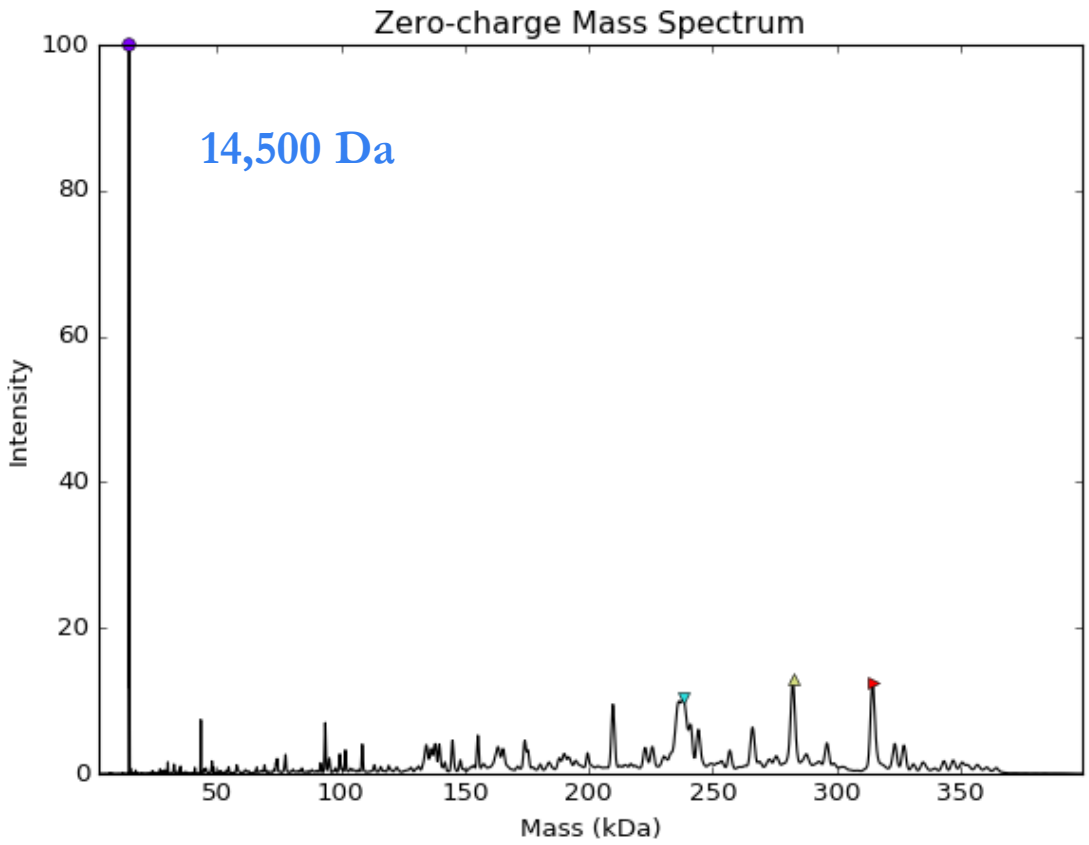
Decamer peak (6 - 6.8 min)





Monomer Peak (7.2 - 7.6 min)





Alpha-synuclein CESI-MS study: Conclusions

- First to demonstrate clear separation of discrete aggregated species of alpha synuclein under native conditions
- Data suggests that starting sample contains a complex mixture of molecular forms
- Method offers the opportunity to study kinetics of alpha-synuclein self-association in detail

Summary

- CESI-MS enables direct coupling of CE with the mass spectrometer in nanospray mode
- Native protein separations offer the ability to study protein conformational states, ligand binding and protein-protein interactions
- Large proteins and protein complexes can be analyzed in contrast to conventional chromatographic & infusion approaches